

Leading articles

Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy

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Originally described in bacteria, drug transporters (or efflux pumps) are now recognized as major determinants in the modulation of the accumulation and efflux of antibacterials in virtually all cell types, from prokaryotes to superior eukaryotes. Transport proteins are in fact major cellular products. Based on sequence similarities with known transporters and with proteins possessing at least two transmembrane segments, it has been calculated that 15–20% of the genome of *Escherichia coli* or of *Saccharomyces cerevisiae* may code for this type of protein.¹ At least 300 gene products are proposed to transport known substrates effectively, out of which ~20–30 transport antibiotics and other drugs.² Figure 1, on this basis, identifies the main groups of transporters (also referred to as superfamilies) that have been shown so far to act effectively upon antibiotics. Two of these superfamilies [major facilitator superfamily (MFS) and ATP binding cassette superfamily (ABC)] span the prokaryote–eukaryote boundary, but with specific members in each kingdom. It must be remembered, however, that most transporters have been identified only very recently, so that the discovery of many more families, with both prokaryotic and eukaryotic members, would not be surprising in the near future. Efflux pumps usually consist of a monocomponent protein with several transmembrane spanning domains (most often 12 of them). However, in Gram-negative bacteria, which are protected by an outer membrane, efflux transporters can be organized as multicomponent systems, in which the efflux pump located in the inner membrane works in conjunction with a periplasmic fusion protein and an outer membrane protein (Figure 2).³ This first review focuses on the impact for antibiotic treatments of efflux pumps found in prokaryotes, while the companion paper⁴ examines those characterized in eukaryotes.

Why antibiotic transporters?

The first description of antibiotic transporters in bacteria resulted from the study of resistance to tetracyclines in the early 1980s.⁵ Soon after, several transporters were identified based on the same approach, i.e. through the unravelling of resistance mechanisms towards various classes of antibacterials, including successively the macrolides,⁶ the fluoroquinolones,⁷ the β -lactams⁸ and, more recently, the aminoglycosides.⁹ This led eventually to the concept that efflux must be considered as a common and basic mechanism of resistance, along the same lines as, and perhaps even more ubiquitous than, target modification or production of antibiotic-inactivating enzymes. The reason why antibiotics are subject to efflux may simply be that most of them share the necessary basic structural features for effective recognition. These determinants are most often the simple combination of an amphipathic character and the presence of an ionizable function. In this context, antibiotics appear as occasional substrates of transporters aimed at protecting cells from exogenous, diffusible molecules. Other examples of potentially harmful substrates include biocides¹⁰ or bile salts for Enterobacteriaceae.¹¹ But efflux pumps can also be viewed as machines developed by cells to extrude poorly diffusible or toxic endogenous molecules from the cytosol, and the latter may constitute some of the natural substrates of these transporters. Using *Pseudomonas aeruginosa* PAO1 wild-type and its efflux mutants, it was shown with a model of epithelial cells and of murine endogenous septicaemia, that most mutants demonstrated significantly reduced invasiveness and decreased capacity to kill mice. Invasiveness was restored by complementation of the mutants with the wild-type genes, or

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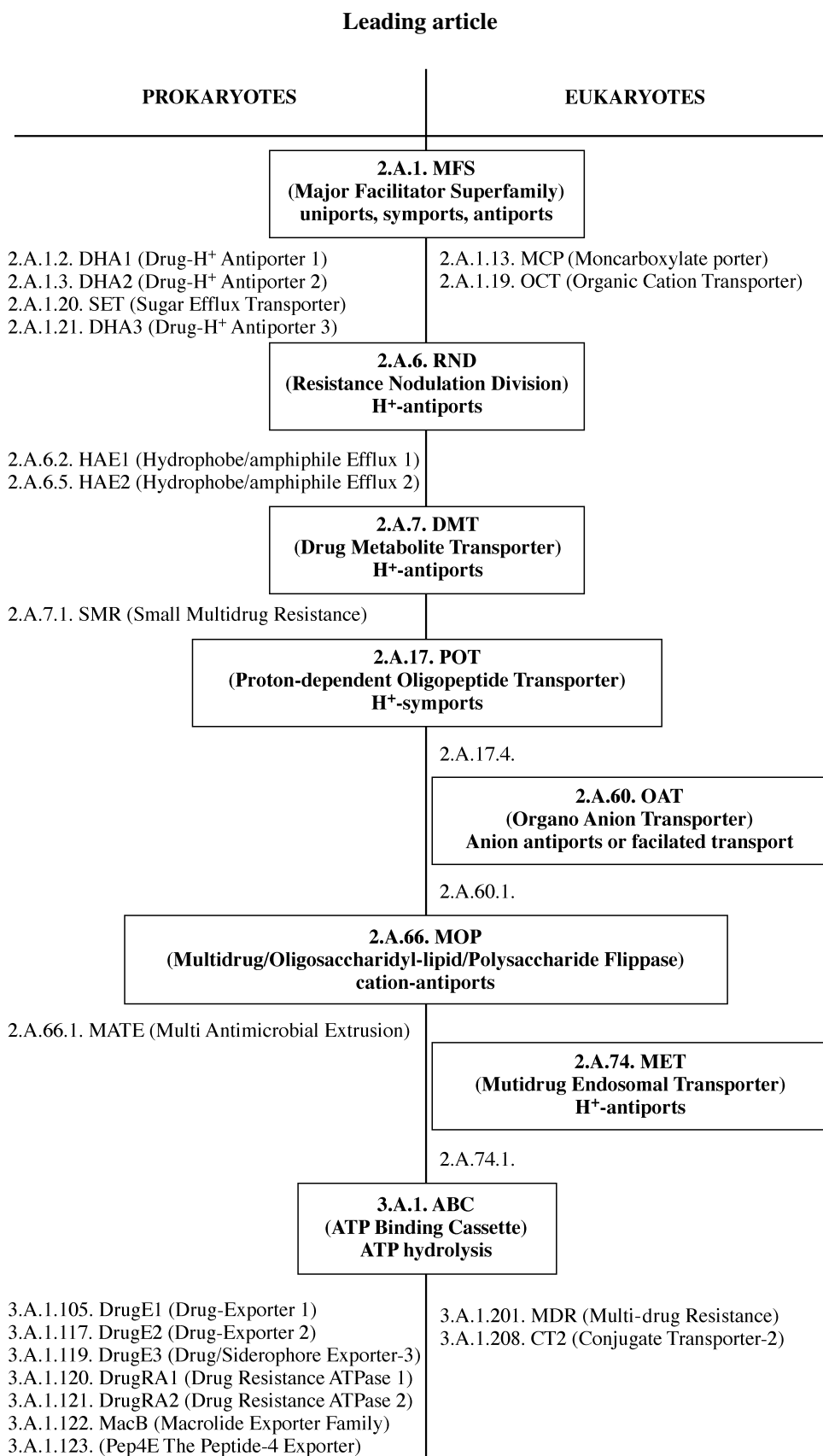


Figure 1. Occurrence and distribution of antibiotic transporters in prokaryotic and eukaryotic cells. The nomenclature is based on the classification of Saier.² The transporters are grouped in so-called superfamilies (three-component nomenclature; bold characters), six of which are found in prokaryotes and eukaryotes. Within each superfamily, only those families (four-component nomenclature; normal characters) transporting antibiotics are shown. OAT and MET are not found in prokaryotes.

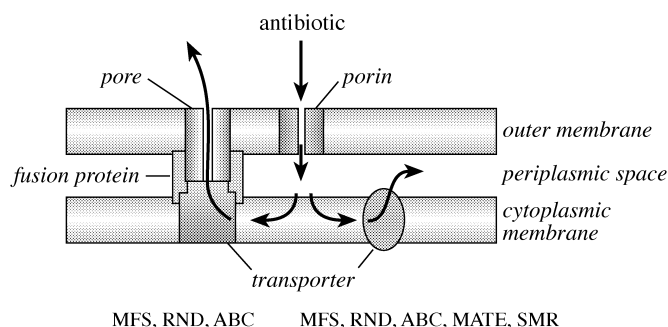


Figure 2. Topology of multicomponent (left) and monocomponent (right) efflux pumps as they can be found in the MFS, RND and ABC superfamilies and in the MFS, RND, ABC and MATE superfamilies, respectively. Multicomponent efflux pumps are specific to Gram-negative bacteria, since their particular organization allows extrusion of antibiotics directly into the extracellular medium. Arrows show directions of antibiotic transport. MFS, RND, SMR and MATE are energized by ion gradients and ABC by ATP hydrolysis.

by adding culture supernatants from epithelial cells infected with wild-type.¹² Along the same lines, the quorum-sensing auto-inducer 3-oxo-C12-homoserine lactone, which plays a key role in *P. aeruginosa* pathogenesis, is regulated by the MexAB–OprM efflux system.¹³ These effects can, however, be counterbalanced by the reduced fitness that may occur as a consequence of the extrusion of bacterial metabolites,¹⁴ or by a decreased intracellular concentration of auto-inducing virulence factors,¹⁵ as shown in MDR-overproducing mutants of *P. aeruginosa*. Finally, combining both self-protecting and physiological roles, transporters also export bacterial toxins¹⁶ as well as antibiotics from antibiotic producers.¹⁷

What are the main antibiotic transporters?

Table 1 shows, in a systematic fashion, the antibiotic transporters positively identified in some important human pathogens. For each of them, we present the necessary elements of identification and a non-exhaustive list of antibiotics transported. Many other efflux pumps are suspected to exist, based either on phylogenetic comparison with established transporters, or on phenotypic characterization (see 18 for a recent reference). Examination of Table 1 shows three major features. First, several transporters recognize very different classes of antibiotics. This holds especially true for resistance nodulation division (RND) superfamily transporters and is the basis for the cross-resistance of several bacteria to apparently structurally unrelated drugs (substituting one class of antibiotics by another will not be a satisfactory answer to resistance detected in these isolates). Secondly, a given species (typically *P. aeruginosa* and *E. coli*) may express quite different drug transporters, leading again to multiresistant phenotypes. Thirdly, a given antibiotic may be recognized by different pumps, and some classes such as tetracyclines,

macrolides, fluoroquinolones and chloramphenicol appear as quasi-universal substrates. However, individual drugs, within a given class, may show specific behaviour with respect to recognition by transporters (see Table 2). This has raised passionate debate concerning apparently closely related molecules, but may simply illustrate the critical impact of apparently minor chemical modifications in the recognition process of substrates by transporters.

Impact on resistance

Generally speaking, efflux mechanisms confer a low to moderate level of resistance only (1- to 64-fold increase in MIC),^{19,20} so that their clinical relevance has been questioned.²¹ The following points need, therefore, to be carefully taken into consideration. First, the intrinsic (or natural) resistance of many bacteria to antibiotics depends on the constitutive or inducible expression of active efflux systems. A typical example is *P. aeruginosa*, which was thought for a long time to be poorly susceptible to a large range of antibiotics of different classes because of the relative impermeability of its outer membrane to drugs. However, the simple disruption of the gene coding for the MexB pump dramatically increases the susceptibility of *P. aeruginosa* to β -lactams, tetracyclines, fluoroquinolones and chloramphenicol.²² Likewise, disruption of the gene coding for MdrL in *Listeria monocytogenes* causes a 10-fold decrease in the MIC of cefotaxime,²³ which suggests that the intrinsic resistance of *L. monocytogenes* to cephalosporins may be due to other mechanisms besides the existence of penicillin-binding proteins with low affinity towards this subclass of β -lactams. Finally, the poor susceptibility of *Haemophilus influenzae* to macrolides may result partly from the presence of an efflux mechanism²⁴ (in addition to the well known effect of acidity on the activity of macrolides, a CO₂-containing atmosphere is necessary to grow this bacteria). This suggests fresh avenues for the design of new macrolides with enhanced activity against *H. influenzae*.

Secondly, concomitant expression of several efflux pumps in a given bacterial species may lead to apparently ‘high level’ resistance phenotypes when considering the shared substrates. This has been observed in Gram-negative bacteria, in which the multicomponent efflux pumps of the RND superfamily transport antibiotics from the cytosolic leaflet of the inner membrane to the periplasmic space, and the single component efflux pumps of the MFS superfamily promote efflux from this space to the external medium.²⁵

Thirdly, efflux may also cooperate with other resistance mechanisms to confer not only high level but also broad-spectrum resistance. For example, the high intrinsic penem resistance of *P. aeruginosa* results from the interplay between the outer membrane barrier, the active efflux system MexAB–OprM and AmpC β -lactamase.²⁶ In *E. coli*, expression of

Table 1. Main efflux transporters as observed in clinically important human pathogens with their corresponding antibiotic substrates^a

Pathogen	Transporter	Super-family	TC number ^b	Antibiotics																	
				β-lactams								Q									
				peni	ceph	carb	m-bac	inhib β-ase	FA	AG	Tet	OX	ML	SG	LM	CHL	RIF	NAL	FQ	SM	TMP
<i>S. aureus</i> <i>S. pneumoniae</i> <i>Streptococcus pyogenes</i> <i>L. monocytogenes</i> <i>Mycobacterium tuberculosis</i> <i>Enterococcus</i> spp. <i>H. influenzae</i> <i>Neisseria gonorrhoeae</i> <i>Salmonella</i> spp. <i>Shigella dysenteriae</i> <i>E. coli</i>	NorA ⁷	MFS	2.A.1.2.10													+ ⁵⁸			+ ⁵⁸		
	TetK-L ⁵⁹	MFS	2.A.1.3.6								+ ³¹										
	MdeA ⁶⁰	MFS								+ ⁶⁰											
	MsrA ⁶	ABC	3.A.1.121.1										+ ⁶								
	MefE ⁶¹	MFS											+ ⁶¹								
	PmrA ⁶²	MFS																	+ ⁶²		
	TetK-L	MFS									+ ³¹										
	MefA ⁶³	MFS	2.A.1.21.2										+ ⁶³		+ ⁶³						
	MdrL ²³	MFS		− ²³	+ ²³					− ²³	− ²³		+ ²³		+ ²³						
	Lde ⁶⁴	MFS																	+ ⁶⁴		
	TetK-L	MFS									+ ³¹										
	Mmr ⁶⁵	SMR	2.A.7.1.2.										+ ⁶⁵								
	TetK-L	MFS									+ ³¹										
	DrrB ⁶⁶	ABC	3.A.1.105.1																+ ⁶⁶		
	Mef ⁶⁷	MFS											+ ⁶⁷								
	TetK-L	MFS									+ ³¹										
	EmeA ⁶⁸	MFS											+ ⁶⁸		+ ⁶⁸				+ ⁶⁸		
	Lsa ⁶⁹	ABC												+ ⁶⁹	+ ⁶⁹						
	TetB, K	MFS									+ ³¹										
	AcrB-like	RND											+ ⁷⁰				+ ⁷⁰				
	MtrD ⁷¹	RND	2.A.6.2.5	+ ⁷²					+ ⁷²		+ ⁷²		+ ⁷²			+ ⁷²	+ ⁷²				
	AcrB ⁷³	RND		+ ⁷⁴	+ ⁷⁴				+ ⁷⁴		+ ⁷⁴		+ ⁷⁴			+ ⁷⁴	+ ⁷⁴	+ ⁷⁴	+ ⁷⁴		
	TetA-D	MFS									+ ³¹										
	FloR ⁷⁵	MFS														+ ⁷⁵					
	TetA-D	MFS									+ ³¹										
	EmrE ⁷⁶	SMR	2.A.7.1.3								+ ⁷⁷		+ ⁷⁷							+ ⁷⁷	
	YdhE ⁷⁸	MATE	2.A.66.1.3												+ ⁷⁹				+ ⁷⁹		+ ⁷⁹
	TetA-E ⁸⁰	MFS	2.A.1.2.4								+ ³¹										
	Bcr ⁸¹	MFS	2.A.1.2.7								+ ⁷⁹									+ ⁸²	
	MdfA ⁸³	MFS	2.A.1.2.19							+ ^{79,83}	+ ⁸³		+ ⁸³			+ ^{79,83}	+ ⁸³		+ ^{79,83}		+ ⁷⁹
	YceL ⁸⁴	MFS	2.A.1.2.21																+ ⁷⁹		
	YidY ⁸⁴	MFS	2.A.1.2.22													+ ⁷⁹					
	EmrB ⁸⁵	MFS	2.A.1.3.2								− ⁸⁵					− ⁸⁵		+ ⁸⁵	− ⁸⁵		
	YebQ ⁸⁴	MFS	2.A.1.3.17																		+ ⁷⁹

[illegible]

^a+, occurrence; -, absence (in both cases, through functional studies).

class-C β -lactamase confers resistance to first- and second-generation cephalosporins, and expression of pump AcrB causes resistance to most penicillins. The global result is that the organism becomes susceptible only to third- or fourth-generation cephalosporins.²⁰ A more indirect but probably very effective mode of cooperation is exemplified by the combination of DNA gyrase and/or topoisomerase IV mutation and efflux in the development of resistance to fluoroquinolones. Whereas single target mutations confer only a low level of resistance, the reduction in the intrabacterial concentration of fluoroquinolones through expression of one or several efflux pumps may result in MICs exceeding break-points (see 27 for a recent example). Thus, resistant strains from clinical sources²⁸ often display a combination of multiple mutations in the target genes and overexpression of efflux transporters.²⁹ More critically, the exposure of the targets to insufficient drug concentrations will favour the selection of mutants. Thus, constitutive expression of efflux pumps acting on fluoroquinolones probably explains the high frequency of mutations leading to resistance in Gram-negative bacteria. This has been particularly well demonstrated for levofloxacin and *P. aeruginosa*. Disruption of the genes of three RND pumps not only brings the MIC from 0.25 to <0.02 mg/L, but, most strikingly, reduces the frequency of appearance of first-step mutants from 2×10^{-7} to $<10^{-11}$.¹⁹ It must, however, be emphasized that all pumps must be inactivated simultaneously to obtain such an effect, since the lack of activity of one can be easily compensated for by overexpression of others with overlapping spectra.³⁰

Fourthly, antibiotics can serve as inducers and regulate the expression of some efflux pumps at the level of gene transcription or mRNA translation, by interacting with regulator systems.³¹ Transporters may also become overexpressed as a result of mutations occurring in these regulators³ (this mechanism may be the predominant one for resistance of *P. aeruginosa* to fluoroquinolones in cystic fibrosis patients).³² More importantly, global regulation may be involved,³³ causing the overexpression of several independent genes (regulons).³⁴ For instance, the *mar* regulon in *E. coli* may control not only the expression of AcrB, but probably also that of other drug-specific transporters and porins, together with numerous other proteins involved in stress responses (see 35 and the references cited therein). Worryingly, mutations in regulator genes might even result in constitutive expression of several efflux pumps, causing multiple resistance.

Fifthly, resistance by efflux can be easily disseminated. In several cases, the genetic elements encoding efflux pumps and their regulators are located on plasmids (such as Tet transporters in Gram-positive bacteria), or on conjugative or transformable transposons located either on plasmids (Tet transporters also, but in Gram-negative bacteria), or in the chromosome (e.g. *mef* genes in *Streptococcus pneumoniae*).³⁶

Table 2. Relative affinities of antibiotics for efflux pumps

Antibiotic class	Affinity for efflux pumps			References
	high	variable ^a	low	
Penicillins ^b	nafcillin, cloxacillin, penicillin G		carbenicillin	74
Cephalosporins ^b	cefalotin, cefotaxime, ceftriaxone		cefazolin, cephaloridin	74
Carbapenems	meropenem	imipenem		98
Macrolides	14- and 15-membered		16-membered, ketolides	107–109
Tetracyclines	tetracycline	minocycline	glycylcyclines ^c	31,110,111
(Fluoro)quinolones	ciprofloxacin, norfloxacin	ofloxacin, levofloxacin	cinafloxacin, gatifloxacin, gemifloxacin, moxifloxacin, ^d garenoxacin	19,112–115

^aDepending on the efflux pump.

^bRanking corresponding to the degree of lipophilicity of the side chain.

^cLow affinity substrate of MexD in *P. aeruginosa* and AcrB and AcrF in *E. coli*.^{116,117}

^dLow affinity substrate of a still unidentified efflux transporter in *S. aureus*.¹¹⁸

Importantly, efflux-mediated resistance mechanisms can spread between phylogenically very distant species. This is exemplified by the macrolide-mediated efflux, which has moved not only among streptococci but also to Gram-negative bacteria.³⁷ Co-transfer with genes for other resistance mechanisms may also take place if these are present together on large mobile genetic elements.

Strategies for the future

Efflux mechanisms should now be taken fully into account in the evaluation of new antibiotics as well as for the future of chemotherapy in the long term. At the level of the clinical microbiology laboratory, suspicion of efflux as a cause of resistance ideally should be confirmed either phenotypically or genotypically so that cross-resistances during therapy can be anticipated. However, phenotypic characterization of efflux may prove difficult or impossible in multiresistant isolates that have several mechanisms. The clear-cut situation of macrolides and streptococci, in which efflux-based resistance is limited to 14- and 15-membered macrolides,³⁸ may be unique. Conversely, demonstrating resistance to a large variety of unrelated agents can suggest the presence of wide-spectrum transporters (see 39 for an example with clinical isolates). Unfortunately, several of the antibiotic substrates recognized by many efflux pump systems (such as chloramphenicol, tetracyclines or trimethoprim) are no longer tested in clinical laboratories because of lack of immediate therapeutic interest. More genotypic characterization could yield important information for surveillance studies. For instance, PCR has been used successfully to identify unambiguously efflux-based resistance mechanisms to macrolides,⁴⁰ tetracyclines⁴¹ and fluoroquinolones,²⁹ and fingerprinting has allowed the spread of the corresponding resistant clones to be

followed.³² Further development of new genotypic tests to improve the characterization of efflux mechanisms in clinical isolates would be welcome in this context (see 42 for first attempts in this direction).

Moving to the evaluation of new antibiotics, exploring their potential recognition by typical efflux transporters must now be in the forefront of their pre-clinical and clinical assessment. All other pharmacological and toxicological properties being equal, the aim here will be to favour the selection of derivatives that are poor substrates of the efflux pumps and that do not induce their overexpression. Table 2 illustrates the relative affinity of current and newly introduced antibiotics for efflux transporters, and reveals that most recent antibiotics in each class are less well recognized than those that are older. The development of pump inhibitors as adjuvant therapy also represents an interesting area for drug discovery, similar to how β -lactamase inhibitors brought new life to β -lactams.⁴³ This approach, however, appears very challenging because of potential effects on efflux transporters also present in eukaryotic cells. It must be emphasized that most inhibitors currently available display strong pharmacological activities in eukaryotic cell systems and are therefore unusable in clinical practice. Typical examples include reserpine,⁴⁴ omeprazole,⁴⁵ phenothiazines,⁴⁶ sertraline,⁴⁷ verapamil⁴⁸ and siderophores.⁴⁹ It is therefore essential to develop molecules not only designed specifically to inhibit prokaryotic transporters, but also with some distance from molecular structures close to those of drugs. Another difficulty is that the pharmacokinetic/dynamic properties of the pump inhibitors will need to match closely those of the companion antibiotic. Only three main categories of compound have been uncovered so far.⁵⁰ The first group comprises compounds specifically raised against the Tet transporters for tetracyclines (namely tetracycline derivatives substituted in position 13 and probably acting as

competitive inhibitors,⁵¹ and a non-competitive inhibitor built on an indan nucleus).⁵² The second group is represented by a family of flavonolignans derived from a natural alkaloid from *Onopordon corymbosum*.⁵³ These molecules inhibit the NorA transporter of *Staphylococcus aureus*. As flavones are also known to inhibit the eukaryotic P-glycoprotein, considerable lead optimization studies will be needed to obtain safe compounds. The third category is represented by peptides acting upon the RND transporters of *P. aeruginosa*.⁵⁴ These inhibitors selectively improve the activity of antibiotics that are substrates of the MexB efflux pump (quinolones, macrolides, chloramphenicol, and to a lesser extent, tetracycline or carbenicillin),⁵⁵ which suggests a high level of specificity.⁵⁶ They do not interact with the eukaryotic P-glycoprotein. A desirable step forward would be to consider the design of wider spectrum inhibitors acting on pumps present in both Gram-positive and Gram-negative bacteria and belonging to different phylogenetic families. Prudent use of these inhibitors will, however, be essential, to avoid fast emergence of resistance to them, which will most probably also emerge. Laboratory mutants of *Bacillus subtilis* have already been isolated with resistance to the inhibitory activity of reserpine.⁵⁷

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References

- Paulsen, I. T., Sliwinski, M. K., Nelissen, B., Goffeau, A. & Saier, M. H., Jr (1998). Unified inventory of established and putative transporters encoded within the complete genome of *Saccharomyces cerevisiae*. *FEBS Letters* **430**, 116–25.
- Saier, M. H., Jr (2002). [Online.] <http://www-biology.ucsd.edu/~msaier/transport/> (19 January 2003, date last accessed).
- Poole, K. (2000). Efflux-mediated resistance to fluoroquinolones in Gram-negative bacteria. *Antimicrobial Agents and Chemotherapy* **44**, 2233–41.
- Van Bambeke, F., Michot, J.-M. & Tulkens, P. M. (2003). Antibiotic efflux pumps in eukaryotic cells: occurrence and impact on antibiotic cellular pharmacokinetics, pharmacodynamics and toxicodynamics. *Journal of Antimicrobial Chemotherapy* **51**, 1067–77.
- McMurry, L., Petrucci, R. E., Jr & Levy, S. B. (1980). Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA* **77**, 3974–7.
- Ross, J. I., Eady, E. A., Cove, J. H., Cunliffe, W. J., Baumberg, S. & Wootton, J. C. (1990). Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Molecular Microbiology* **4**, 1207–14.
- Yoshida, H., Bogaki, M., Nakamura, S., Ubukata, K. & Konno, M. (1990). Nucleotide sequence and characterization of the *Staphylococcus aureus* *norA* gene, which confers resistance to quinolones. *Journal of Bacteriology* **172**, 6942–9.
- Li, X. Z., Ma, D., Livermore, D. M. & Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: active efflux as a contributing factor to beta-lactam resistance. *Antimicrobial Agents and Chemotherapy* **38**, 1742–52.
- Aires, J. R., Kohler, T., Nikaido, H. & Plesiat, P. (1999). Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. *Antimicrobial Agents and Chemotherapy* **43**, 2624–8.
- Poole, K. (2002). Mechanisms of bacterial biocide and antibiotic resistance. *Journal of Applied Microbiology* **92**, Suppl., 55S–64S.
- Thanassi, D. G., Cheng, L. W. & Nikaido, H. (1997). Active efflux of bile salts by *Escherichia coli*. *Journal of Bacteriology* **179**, 2512–8.
- Hirakata, Y., Srikumar, R., Poole, K., Gotoh, N., Suematsu, T., Kohno, S. *et al.* (2002). Multidrug efflux systems play an important role in the invasiveness of *Pseudomonas aeruginosa*. *Journal of Experimental Medicine* **196**, 109–18.
- Rahmati, S., Yang, S., Davidson, A. L. & Zechiedrich, E. L. (2002). Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. *Molecular Microbiology* **43**, 677–85.
- Sanchez, P., Linares, J. F., Ruiz-Diez, B., Campanario, E., Navas, A., Baquero, F. *et al.* (2002). Fitness of *in vitro* selected *Pseudomonas aeruginosa* *nalB* and *nfxB* multidrug resistant mutants. *Journal of Antimicrobial Chemotherapy* **50**, 657–64.
- Kohler, T., van Delden, C., Curty, L. K., Hamzehpour, M. M. & Pechere, J. C. (2001). Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *Journal of Bacteriology* **183**, 5213–22.
- Sharff, A., Fanutti, C., Shi, J., Calladine, C. & Luisi, B. (2001). The role of the TolC family in protein transport and multidrug efflux. From stereochemical certainty to mechanistic hypothesis. *European Journal of Biochemistry* **268**, 5011–26.
- Cundliffe, E. (1992). Self-protection mechanisms in antibiotic producers. *Ciba Foundation Symposium* **171**, 199–208.
- Piddock, L. J., Jin, Y. F., Webber, M. A. & Everett, M. J. (2002). Novel ciprofloxacin-resistant, nalidixic acid-susceptible mutant of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **46**, 2276–8.
- Lomovskaya, O., Lee, A., Hoshino, K., Ishida, H., Mistry, A., Warren, M. S. *et al.* (1999). Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **43**, 1340–6.
- Mazzariol, A., Cornaglia, G. & Nikaido, H. (2000). Contributions of the AmpC beta-lactamase and the AcrAB multidrug efflux system in intrinsic resistance of *Escherichia coli* K-12 to beta-lactams. *Antimicrobial Agents and Chemotherapy* **44**, 1387–90.
- Lynch, J. P., III & Martinez, F. J. (2002). Clinical relevance of macrolide-resistant *Streptococcus pneumoniae* for community-acquired pneumonia. *Clinical Infectious Diseases* **34**, Suppl. 1, S27–46.

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22. Li, X. Z., Nikaido, H. & Poole, K. (1995). Role of mexA-mexB-oprM in antibiotic efflux in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **39**, 1948–53.
23. Mata, M. T., Baquero, F. & Perez-Diaz, J. C. (2000). A multidrug efflux transporter in *Listeria monocytogenes*. *FEMS Microbiology Letters* **187**, 185–8.
24. Bozdogan, B., Peric, M., Jacobs, M. R. & Appelbaum, P. C. (2002). Macrolide hyper-susceptibility is associated with the absence of efflux mechanism in clinical isolates of *H. influenzae*. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C1-1612, p. 76. American Society for Microbiology, Washington, DC, USA.
25. Lomovskaya, O. (2002). Interactions among multiple efflux pumps: additive and synergistic effects on antimicrobial resistance. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract 1188, p. 466. American Society for Microbiology, Washington, DC, USA.
26. Okamoto, K., Gotoh, N. & Nishino, T. (2001). *Pseudomonas aeruginosa* reveals high intrinsic resistance to penem antibiotics: penem resistance mechanisms and their interplay. *Antimicrobial Agents and Chemotherapy* **45**, 1964–71.
27. Le Thomas, I., Couetdic, G., Clermont, O., Brahimi, N., Plesiat, P. & Bingen, E. (2001). *In vivo* selection of a target/efflux double mutant of *Pseudomonas aeruginosa* by ciprofloxacin therapy. *Journal of Antimicrobial Chemotherapy* **48**, 553–5.
28. Yoshida, T., Muratani, T., Iyobe, S. & Mitsuhashi, S. (1994). Mechanisms of high-level resistance to quinolones in urinary tract isolates of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **38**, 1466–9.
29. Jalal, S. & Wretling, B. (1998). Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aeruginosa*. *Microbial Drug Resistance* **4**, 257–61.
30. Li, X. Z., Barre, N. & Poole, K. (2000). Influence of the MexA-MexB-OprM multidrug efflux system on expression of the MexC-MexD-OprJ and MexE-MexF-OprN multidrug efflux systems in *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy* **46**, 885–93.
31. Roberts, M. C. (1996). Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews* **19**, 1–24.
32. Jalal, S., Ciofu, O., Hoiby, N., Gotoh, N. & Wretling, B. (2000). Molecular mechanisms of fluoroquinolone resistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Antimicrobial Agents and Chemotherapy* **44**, 710–2.
33. Pumbwe, L. & Piddock, L. J. (2000). Two efflux systems expressed simultaneously in multidrug-resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **44**, 2861–4.
34. George, A. M. (1996). Multidrug resistance in enteric and other Gram-negative bacteria. *FEMS Microbiology Letters* **139**, 1–10.
35. Barbosa, T. M. & Levy, S. B. (2000). Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. *Journal of Bacteriology* **182**, 3467–74.
36. Del Grosso, M., Iannelli, F., Messina, C., Santagati, M., Petrosillo, N., Stefani, S. *et al.* (2002). Macrolide efflux genes *mef(A)* and *mef(E)* are carried by different genetic elements in *Streptococcus pneumoniae*. *Journal of Clinical Microbiology* **40**, 774–8.
37. Luna, V. A., Cousin, S., Jr, Whittington, W. L. & Roberts, M. C. (2000). Identification of the conjugative *mef* gene in clinical *Acinetobacter junii* and *Neisseria gonorrhoeae* isolates. *Antimicrobial Agents and Chemotherapy* **44**, 2503–6.
38. Farrell, D. J., Morrissey, I., Bakker, S. & Felmingham, D. (2002). Molecular characterization of macrolide resistance mechanisms among *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolated from the PROTEKT 1999–2000 study. *Journal of Antimicrobial Chemotherapy* **50**, Suppl. S1, 39–47.
39. Zihra-Zarifi, I., Llanes, C., Kohler, T., Pechere, J. C. & Plesiat, P. (1999). *In vivo* emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. *Antimicrobial Agents and Chemotherapy* **43**, 287–91.
40. Sutcliffe, J., Grebe, T., Tait-Kamradt, A. & Wondrack, L. (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrobial Agents and Chemotherapy* **40**, 2562–6.
41. Aminov, R. I., Chee-Sanford, J. C., Garrigues, N., Teferedegne, B., Krapac, I. J., White, B. A. *et al.* (2002). Development, validation, and application of PCR primers for detection of tetracycline efflux genes of Gram-negative bacteria. *Applied and Environmental Microbiology* **68**, 1786–93.
42. Prudencio, C., Sansonetty, F., Sousa, M. J., Corte-Real, M. & Leao, C. (2000). Rapid detection of efflux pumps and their relation with drug resistance in yeast cells. *Cytometry* **39**, 26–35.
43. Ryan, B. M., Dougherty, T. J., Beaulieu, D., Chuang, J., Dougherty, B. A. & Barrett, J. F. (2001). Efflux in bacteria: what do we really know about it? *Expert Opinion on Investigational Drugs* **10**, 1409–22.
44. Brenwald, N. P., Gill, M. J. & Wise, R. (1997). The effect of reserpine, an inhibitor of multi-drug efflux pumps, on the *in-vitro* susceptibilities of fluoroquinolone-resistant strains of *Streptococcus pneumoniae* to norfloxacin. *Journal of Antimicrobial Chemotherapy* **40**, 458–60.
45. Aeschlimann, J. R., Dresser, L. D., Kaatz, G. W. & Rybak, M. J. (1999). Effects of NorA inhibitors on *in vitro* antibacterial activities and postantibiotic effects of levofloxacin, ciprofloxacin, and norfloxacin in genetically related strains of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **43**, 335–40.
46. Molnar, J., Hever, A., Fakla, I., Fischer, J., Ocsovski, I. & Aszalos, A. (1997). Inhibition of the transport function of membrane proteins by some substituted phenothiazines in *E. coli* and multidrug resistant tumor cells. *Anticancer Research* **17**, 481–6.
47. Munoz-Bellido, J. L., Munoz-Criado, S. & Garcia-Rodriguez, J. A. (2000). Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors. *International Journal of Antimicrobial Agents* **14**, 177–80.
48. Cohn, R. C., Rudzienski, L. & Putnam, R. W. (1995). Verapamil-tobramycin synergy in *Pseudomonas cepacia* but not *Pseudomonas aeruginosa* *in vitro*. *Chemotherapy* **41**, 330–3.
49. Rothstein, D. M., McGlynn, M., Bernan, V., McGahren, J., Zaccardi, J., Cekleniak, N. *et al.* (1993). Detection of tetracyclines and efflux pump inhibitors. *Antimicrobial Agents and Chemotherapy* **37**, 1624–9.

Leading article

50. Lomovskaya, O. & Watkins, W. J. (2001). Efflux pumps: their role in antibacterial drug discovery. *Current Medicinal Chemistry* **8**, 1699–711.
51. Nelson, M. L. & Levy, S. B. (1999). Reversal of tetracycline resistance mediated by different bacterial tetracycline resistance determinants by an inhibitor of the Tet(B) antiport protein. *Antimicrobial Agents and Chemotherapy* **43**, 1719–24.
52. Hirata, T., Wakatabe, R., Nielsen, J., Satoh, T., Nihira, S. & Yamaguchi, A. (1998). Screening of an inhibitor of the tetracycline efflux pump in a tetracycline-resistant clinical isolate of *Staphylococcus aureus* 743. *Biological and Pharmaceutical Bulletin* **21**, 678–81.
53. Guz, N. R., Stermitz, F. R., Johnson, J. B., Beeson, T. D., Willen, S., Hsiang, J. *et al.* (2001). Flavonolignan and flavone inhibitors of a *Staphylococcus aureus* multidrug resistance pump: structure–activity relationships. *Journal of Medicinal Chemistry* **44**, 261–8.
54. Renau, T. E., Leger, R., Flamme, E. M., Sangalang, J., She, M. W., Yen, R. *et al.* (1999). Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of the fluoroquinolone antibacterial levofloxacin. *Journal of Medicinal Chemistry* **42**, 4928–31.
55. Lomovskaya, O., Warren, M. S., Lee, A., Galazzo, J., Fronko, R., Lee M. *et al.* (2001). Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrobial Agents and Chemotherapy* **45**, 105–16.
56. Barrett, J. F. (2001). MC-207110 Daiichi Seiyaku/Microcide Pharmaceuticals. *Current Opinion on Investigational Drugs* **2**, 212–5.
57. Ahmed, M., Borsch, C. M., Neyfakh, A. A. & Schuldiner, S. (1993). Mutants of the *Bacillus subtilis* multidrug transporter Bmr with altered sensitivity to the antihypertensive alkaloid reserpine. *Journal of Biological Chemistry* **268**, 11086–9.
58. Neyfakh, A. A., Borsch, C. M. & Kaatz, G. W. (1993). Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrobial Agents and Chemotherapy* **37**, 128–9.
59. Noguchi, N., Aoki, T., Sasatsu, M., Kono, M., Shishido, K. & Ando, T. (1986). Determination of the complete nucleotide sequence of pNS1, a staphylococcal tetracycline-resistance plasmid propagated in *Bacillus subtilis*. *FEMS Microbiology Letters* **37**, 283–8.
60. Huang, J., O'Toole P. W., Shen, W., Jiang, X., Lobo, N., Palmer, L. M. *et al.* (2002). Identification of a novel multidrug resistance efflux system (MdeA) from *Staphylococcus aureus*. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C1-427, p. 65. American Society for Microbiology, Washington, DC, USA.
61. Tait-Kamradt, A., Clancy, J., Cronan, M., Dib-Hajj, F., Wondrack, L., Yuan, W. *et al.* (1997). *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **41**, 2251–5.
62. Gill, M. J., Brenwald, N. P. & Wise, R. (1999). Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **43**, 187–9.
63. Clancy, J., Petitpas, J., Dib-Hajj, F., Yuan, W., Cronan, M., Kamath, A. V. *et al.* (1996). Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. *Molecular Microbiology* **22**, 867–79.
64. Godreuil, S., Galimand, M., Gerbaud, G. & Courvalin, P. (2001). Efflux pump Lde is associated with fluoroquinolone resistance in *Listeria monocytogenes* CLIP 21369. In *Program and Abstracts of the Forty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, USA, 2001*. Abstract UL-11. American Society for Microbiology, Washington, DC, USA.
65. De Rossi, E., Branzoni, M., Cantoni, R., Milano, A., Riccardi, G. & Ciferri, O. (1998). *mmr*, a *Mycobacterium tuberculosis* gene conferring resistance to small cationic dyes and inhibitors. *Journal of Bacteriology* **180**, 6068–71.
66. Chaudhuri, B. S., Bhakta, S., Barik, R., Basu, J., Kundu, M. & Chakrabarti, P. (2002). Overexpression and functional characterization of an ABC transporter encoded by the genes *ddrA* and *ddrB* of *Mycobacterium tuberculosis*. *Biochemical Journal* **367**, 279–85.
67. Luna, V. A., Coates, P., Eady, E. A., Cove, J. H., Nguyen, T. T. & Roberts, M. C. (1999). A variety of Gram-positive bacteria carry mobile *mef* genes. *Journal of Antimicrobial Chemotherapy* **44**, 19–25.
68. Jonas, B. M., Murray, B. E. & Weinstock, G. M. (2001). Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. *Antimicrobial Agents and Chemotherapy* **45**, 3574–9.
69. Singh, K. V., Weinstock, G. M. & Murray, B. E. (2002). An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. *Antimicrobial Agents and Chemotherapy* **46**, 1845–50.
70. Sanchez, L., Pan, W., Vinas, M. & Nikaido, H. (1997). The *acrAB* homolog of *Haemophilus influenzae* codes for a functional multidrug efflux pump. *Journal of Bacteriology* **179**, 6855–7.
71. Hagman, K. E., Lucas, C. E., Balthazar, J. T., Snyder, L., Nilles, M., Judd, R. C. *et al.* (1997). The MtrD protein of *Neisseria gonorrhoeae* is a member of the resistance/nodulation/division protein family constituting part of an efflux system. *Microbiology* **143**, 2117–25.
72. Nikaido, H. (1998). Antibiotic resistance caused by Gram-negative multidrug efflux pumps. *Clinical Infectious Diseases* **27**, Suppl. 1, S32–41.
73. Lacroix, F. J., Cloeckert, A., Grepinet, O., Pinault, C., Popoff, M. Y., Waxin, H. *et al.* (1996). *Salmonella typhimurium* *acrB*-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. *FEMS Microbiology Letters* **135**, 161–7.
74. Nikaido, H., Basina, M., Nguyen, V. & Rosenberg, E. Y. (1998). Multidrug efflux pump AcrAB of *Salmonella typhimurium* excretes only those beta-lactam antibiotics containing lipophilic side chains. *Journal of Bacteriology* **180**, 4686–92.
75. Arcangioli, M. A., Leroy-Setrin, S., Martel, J. L. & Chaslus-Dancla, E. (1999). A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in *Salmonella typhimurium* DT104. *FEMS Microbiology Letters* **174**, 327–32.
76. Morimyo, M., Hongo, E., Hama-Inaba, H. & Machida, I. (1992). Cloning and characterization of the *mvrC* gene of *Escherichia coli*

Leading article

K-12 which confers resistance against methyl viologen toxicity. *Nucleic Acids Research* **20**, 3159–65.

77. Yerushalmi, H., Lebendiker, M. & Schuldiner, S. (1995). EmrE, an *Escherichia coli* 12-kDa multidrug transporter, exchanges toxic cations and H⁺ and is soluble in organic solvents. *Journal of Biological Chemistry* **270**, 6856–63.

78. Morita, Y., Kodama, K., Shiota, S., Mine, T., Kataoka, A., Mizushima, T. *et al.* (1998). NorM, a putative multidrug efflux protein, of *Vibrio parahaemolyticus* and its homolog in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **42**, 1778–82.

79. Nishino, K. & Yamaguchi, A. (2001). Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. *Journal of Bacteriology* **183**, 5803–12.

80. Waters, S. H., Rogowsky, P., Grinsted, J., Altenbuchner, J. & Schmitt, R. (1983). The tetracycline resistance determinants of RP1 and Tn1721: nucleotide sequence analysis. *Nucleic Acids Research* **11**, 6089–105.

81. Bentley, J., Hyatt, L. S., Ainley, K., Parish, J. H., Herbert, R. B. & White, G. R. (1993). Cloning and sequence analysis of an *Escherichia coli* gene conferring bicyclomycin resistance. *Gene* **127**, 117–20.

82. Vedantam, G., Guay, G. G., Austria, N. E., Doktor, S. Z. & Nichols, B. P. (1998). Characterization of mutations contributing to sulfathiazole resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **42**, 88–93.

83. Edgar, R. & Bibi, E. (1997). MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. *Journal of Bacteriology* **179**, 2274–80.

84. Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M. *et al.* (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–74.

85. Lomovskaya, O. & Lewis, K. (1992). Emr, an *Escherichia coli* locus for multidrug resistance. *Proceedings of the National Academy of Sciences, USA* **89**, 8938–42.

86. Yura, T., Mori, H., Nagai, H., Nagata, T., Ishihama, A., Fujita, N. *et al.* (1992). Systematic sequencing of the *Escherichia coli* genome: analysis of the 0–2.4 min region. *Nucleic Acids Research* **20**, 3305–8.

87. Saier, M. H., Jr (2000). Families of transmembrane sugar transport proteins. *Molecular Microbiology* **35**, 699–710.

88. Fujisaki, S., Ohnuma, S., Horiuchi, T., Takahashi, I., Tsukui, S., Nishimura, Y. *et al.* (1996). Cloning of a gene from *Escherichia coli* that confers resistance to fosmidomycin as a consequence of amplification. *Gene* **175**, 83–7.

89. Ma, D., Cook, D. N., Alberti, M., Pon, N. G., Nikaido, H. & Hearst, J. E. (1993). Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. *Journal of Bacteriology* **175**, 6299–313.

90. Okusu, H., Ma, D. & Nikaido, H. (1996). AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. *Journal of Bacteriology* **178**, 306–8.

91. Buysse, J. M., Demyan, W. F., Dunyak, D. S., Stapert, D., Hamel, J. C. & Ford, C. W. (1996). Mutation of the AcrAB antibiotic efflux pump in *Escherichia coli* confers susceptibility to oxazolidinone antibiotics. In *Program and Abstracts of the Thirty-sixth Inter-*

science Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, USA, 1996. Abstract C-42. American Society for Microbiology, Washington, DC, USA.

92. Rosenberg, E. Y., Ma, D. & Nikaido, H. (2000). AcrD of *Escherichia coli* is an aminoglycoside efflux pump. *Journal of Bacteriology* **182**, 1754–6.

93. Kobayashi, N., Nishino, K. & Yamaguchi, A. (2001). Novel macrolide-specific ABC-type efflux transporter in *Escherichia coli*. *Journal of Bacteriology* **183**, 5639–44.

94. Alonso, A. & Martinez, J. L. (2000). Cloning and characterization of SmeDEF, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* **44**, 3079–86.

95. Zhang, L., Li, X. Z. & Poole, K. (2001). SmeDEF multidrug efflux pump contributes to intrinsic multidrug resistance in *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* **45**, 3497–503.

96. Bissonnette, L., Champetier, S., Buisson, J. P. & Roy, P. H. (1991). Characterization of the nonenzymatic chloramphenicol resistance (*cmIA*) gene of the *ln4* integron of Tn1696: similarity of the product to transmembrane transport proteins. *Journal of Bacteriology* **173**, 4493–502.

97. Poole, K., Heinrichs, D. E. & Neshat, S. (1993). Cloning and sequence analysis of an *EnvCD* homologue in *Pseudomonas aeruginosa*: regulation by iron and possible involvement in the secretion of the siderophore pyoverdine. *Molecular Microbiology* **10**, 529–44.

98. Kohler, T., Michea-Hamzehpour, M., Epp, S. F. & Pechere, J. C. (1999). Carbapenem activities against *Pseudomonas aeruginosa*: respective contributions of OprD and efflux systems. *Antimicrobial Agents and Chemotherapy* **43**, 424–7.

99. Masuda, N., Gotoh, N., Ishii, C., Sakagawa, E., Ohya, S. & Nishino, T. (1999). Interplay between chromosomal beta-lactamase and the MexAB-OprM efflux system in intrinsic resistance to beta-lactams in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **43**, 400–2.

100. Li, X. Z., Zhang, L., Srikumar, R. & Poole, K. (1998). Beta-lactamase inhibitors are substrates for the multidrug efflux pumps of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **42**, 399–403.

101. Masuda, N., Sakagawa, E., Ohya, S., Gotoh, N., Tsujimoto, H. & Nishino, T. (2000). Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **44**, 3322–7.

102. Poole, K., Gotoh, N., Tsujimoto, H., Zhao, Q., Wada, A., Yamasaki, T. *et al.* (1996). Overexpression of the *mexC-mexD-oprJ* efflux operon in *nfxB*-type multidrug-resistant strains of *Pseudomonas aeruginosa*. *Molecular Microbiology* **21**, 713–24.

103. Kohler, T., Michea-Hamzehpour, M., Henze, U., Gotoh, N., Curty, L. K. & Pechere, J. C. (1997). Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. *Molecular Microbiology* **23**, 345–54.

104. Maseda, H., Yoneyama, H. & Nakae, T. (2000). Assignment of the substrate-selective subunits of the MexEF-OprN multidrug efflux pump of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **44**, 658–64.

105. Chuanchuen, R., Narasaki, C. T. & Schweizer, H. P. (2002). The MexJK efflux pump of *Pseudomonas aeruginosa* requires

Leading article

OprM for antibiotic efflux but not for efflux of triclosan. *Journal of Bacteriology* **184**, 5036–44.

106. Mine, T., Morita, Y., Kataoka, A., Mizushima, T. & Tsuchiya, T. (1999). Expression in *Escherichia coli* of a new multidrug efflux pump, MexXY, from *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **43**, 415–7.

107. Clancy, J., Dib-Hajj, F., Petitpas, J. W. & Yuan, W. (1997). Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae*. *Antimicrobial Agents and Chemotherapy* **41**, 2719–23.

108. Capobianco, J. O., Cao, Z., Shortridge, V. D., Ma, Z., Flamm, R. K. & Zhong, P. (2000). Studies of the novel ketolide ABT-773: transport, binding to ribosomes, and inhibition of protein synthesis in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **44**, 1562–7.

109. Giovanetti, E., Montanari, M. P., Marchetti, F. & Varaldo, P. E. (2000). *In vitro* activity of ketolides telithromycin and HMR 3004 against Italian isolates of *Streptococcus pyogenes* and *Streptococcus pneumoniae* with different erythromycin susceptibility. *Journal of Antimicrobial Chemotherapy* **46**, 905–8.

110. Someya, Y., Yamaguchi, A. & Sawai, T. (1995). A novel glycylicycline, 9-(N,N-dimethylglycylamido)-6-demethyl-6-deoxytetracycline, is neither transported nor recognized by the transposon Tn10-encoded metal-tetracycline/H⁺ antiporter. *Antimicrobial Agents and Chemotherapy* **39**, 247–9.

111. Mendez, B., Tachibana, C. & Levy, S. B. (1980). Heterogeneity of tetracycline resistance determinants. *Plasmid* **3**, 99–108.

112. Piddock, L. J., Johnson, M., Ricci, V. & Hill, S. L. (1998). Activities of new fluoroquinolones against fluoroquinolone-resistant pathogens of the lower respiratory tract. *Antimicrobial Agents and Chemotherapy* **42**, 2956–60.

113. Heaton, V. J., Goldsmith, C. E., Ambler, J. E. & Fisher, L. M. (1999). Activity of gemifloxacin against penicillin- and ciprofloxacin-

resistant *Streptococcus pneumoniae* displaying topoisomerase- and efflux-mediated resistance mechanisms. *Antimicrobial Agents and Chemotherapy* **43**, 2998–3000.

114. Takenouchi, T., Tabata, F., Iwata, Y., Hanzawa, H., Sugawara, M. & Ohya, S. (1996). Hydrophilicity of quinolones is not an exclusive factor for decreased activity in efflux-mediated resistant mutants of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **40**, 1835–42.

115. Schmitz, F. J., Boos, M., Mayer, S., Kohrer, K., Scheuring, S. & Fluit, A. C. (2002). *In vitro* activities of novel des-fluoro(6) quinolone BMS-284756 against mutants of *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus* selected with different quinolones. *Antimicrobial Agents and Chemotherapy* **46**, 934–5.

116. Dean, C. R., Visalli, M. A., Projan, S. J., Sum, P. E. & Bradford, P. A. (2002). Efflux mediated resistance to tigecycline in *Pseudomonas aeruginosa*. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C1-1601, p. 73. American Society for Microbiology, Washington, DC, USA.

117. Hirata, T., Nishino, K., Saito, A., Tamura, N. & Yamaguchi, A. (2002). Characterization of a novel tetracycline derivative, tigecycline (GAR-936) as a substrate for efflux proteins: potential for developing resistance in *E. coli* strains. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C1-1602, p. 74. American Society for Microbiology, Washington, DC, USA.

118. Kaatz, G. W., Moudgal, V. V. & Seo, S. M. (2002). Multidrug efflux phenotype in *Staphylococcus aureus* conferred by a pump recognizing organic cations and C8-methoxy fluoroquinolones. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C1-425, p. 64. American Society for Microbiology, Washington, DC, USA.

